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The genus *Podaxis* in arid regions of Mexico: preliminary ITS phylogeny and ethnomycological use

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Abstract

Identification of *Podaxis* species to species-level based on morphology alone is problematic. Thus, species of the genus *Podaxis* are in dire need of taxonomic and phylogenetic evaluations using molecular data to develop a consensus between morphological taxonomy and more robust molecular analyses. In Mexico, most of the collected specimens of *Podaxis* have been morphologically identified as *Podaxis pistillaris sensu lato* and are locally used for its culinary value. In this study, the internal transcribed spacer region of *Podaxis* specimens from the MEXU fungarium collected between 1948 and 2014 from arid regions of Mexico were sequenced and these collections placed into a molecular phylogenetic framework using Maximum Likelihood analysis. In addition, the ethnomycological use of *Podaxis* in Mexico (utility, traditional handling, economic importance, etc.) is described by observations, interviews, and sampling of *Podaxis* species with local people from three areas of the region of the Cañada of Oaxaca, which belongs to the Tehuacán-Cuicatlán Biosphere Reserve. These results indicate that the Mexican *Podaxis* were divided into two clades. Specimens collected in the northern region showed phylogenetic affinities to clade D, while specimens from the south of Mexico clustered within clade E. Morphological data, such as spore length and width, showed significant differences between the two phylogenetic clades, implying that these clades represent different species. None of the Mexican specimens were found in association with termite mounds, which might indicate an adaptation to desert-like regions. This study provides the first ethnomycological use of *Podaxis* from Mexico.

Key words

Basidiomycota, edible mushroom, *Podaxis pistillaris*

Introduction

Podaxis has been collected from numerous arid regions around world; approximately 44 species have been described to date (Conlon et al. 2016). This genus encompasses a wide range of morphological characters such as variation in color, size and shapes in fruit body morphology, as well as a wide range of spore length, width and wall thickness, and has often been confused with *Coprinus comatus* (Morse 1933; Morse 1941; Herrera 1950). Earlier classifications have placed *Podaxis* within the family Podaxaceae (Morse 1933); however, modern taxonomic classification places it within the family Agaricaceae (Kirk et al. 2008). Recently, Conlon and collaborators (2016) studied 45 specimens labeled as *Podaxis pistillaris*, mainly from South Africa, and based on combined internal transcribed spacer (ITS) region and LSU rDNA phylogeny analyses demonstrated that the genus contained at least six clades (A-F) representing different putative *Podaxis* spp.

In Mexico, *Podaxis* was reported for the first time in 1893 as *P. mexicanus* from Agiabampo, Sonora (Ellis 1893). Then, in 1908, N. T. Patouillard identified *P. farlowii* also from Sonora (Morse 1933), and in 1938, D. H. Linder identified *P. farlowii* from Hipolito, Coahuila (http://mycoportal.org). Since then, all the specimens, including the ones deposited at the fungarium of the Herbario Nacional de Mexico (MEXU), have been described as *P. pistillaris* (Herrera 1950; Guzmán and Herrera 1969; Guzmán and Herrera 1973; Urista et al. 1985; Esqueda et al. 2010; Esqueda et al. 2012). The reduction of names of all specimens in the MEXU fungarium to *P. pistillaris* has not been previously investigated in light of molecular data.

Despite the occurrence of *Podaxis* in arid regions of Mexico, the ethnomycological use of this mushroom in the country is undocumented. This is particularly important since *Podaxis* spp. have been widely utilized for its culinary value by indigenous people, particularly in the Tehuacán-Cuicatlán Biosphere Reserve (RBTC) in the south of Mexico. In this context, the goals of this study were: 1) to analyze via ITS sequencing newly collected and fungarium specimens of *Podaxis* from Mexico to better predict their molecular phylogenetic placement and thus establish if one or more phylogenetic species of *Podaxis* exist in Mexico; and 2) to describe the traditional use, handling and economic importance of *Podaxis* spp. in the RBTC by observations and interviews with the local people.

Methods

Fungal material

Eighteen fungarium and five fresh *Podaxis* specimens from different arid regions of Mexico were used for the phylogenetic study. The fresh fruiting bodies were obtained from four sites in three communities of the state of Oaxaca (Table 1); all collections were made during rainy season. Sampling, description, digitalization, and drying of mushrooms were performed as recommended by Cifuentes et al. (1986). We analyzed the specimens in the laboratory, and measured macro and microscopic characteristics

Table 1. Voucher specimens in the fungi collection of the Herbario Nacional de México (MEXU) at the Instituto de Biología, Universidad Nacional Autónoma de México.

Voucher (MEXU)	Clade	Locality	Collector and collection date (mm/dd/yyyy)	Location geography	Type of vegetation	Habitat	Native language	GenBank
1191	D	Oaxaca RBTC	T. Herrera, M. Ruiz-Oronoz (10/16/1948)	San Pedro Chicozapotes, San Juan Bautista Cuicatlán municipality, 633 masl, 17°46.232'N, 96°57.209'W	TrDF	Sandy soil	Cuicatec	KY034680
10805	D	Oaxaca	A. Solís-Magallanes (07/1976)	Presa Benito Juárez, Oaxaca-Tehuantepec	--	Limestone soil	--	KY034681
12808	D	Oaxaca	O. Téllez, M. Sousa, L. Rico (02/20/1978)	Salina Cruz-Pochutla, Salina Cruz, 20 masl	TrDF	--	--	KY034682
7023	D	Oaxaca	T. Herrera (08/04/1979)	Istmo of Tehuantepec	--	Sandy soil	--	KY034683
27558	D	Oaxaca RBTC	A. Medina-Ortiz (06/24/2009)	El Brujo, Santa María Tecomavaca municipality, 626 masl, 17°57.501'N, 97°1.266'W	TrDF	Sandy soil, sandy clay in stony, clayey silt, and cultivation soil	Mazatec and Mixtec	KY034684
27845	D	Oaxaca RBTC	A. Medina-Ortiz, A. de la Cruz-Martínez (10/07/2013)	Santiago Quiotepec, San Juan Bautista Cuicatlán municipality, 626 masl, 17°57.501'N, 97°1.266'W	TrDF and DS	Sandy clay	Cuicatec	KY034686
27557	D	Oaxaca RBTC	A. Medina-Ortiz, E. Pérez-Silva, A. García-Mendoza (07/12/2014)	Cuicatlán-Concepción Pápalo, San Juan Bautista Cuicatlán municipality. 630 masl, 17°47.727'N, 96°57.530'W	TrDF and DS	Sandy soil	Cuicatec	KY034687
5772	D	Durango	J. Sánchez (09/10/1966)	Estación Chocolate, Lerdo, Durango-Torreón	DS	--	--	KY034678
12338	D	Baja California Sur	E. Pérez-Silva 09/01/ 1978	Econhotel, La Paz	--	--	--	KY034673
5015	D	Tamaulipas	A. Gómez-Pompa, E. Nebling (09/03/1967)	Mante-Gonzáles City	ThDF	Clay soil	--	KY034689
7212	D	Tamaulipas	A. Marino (03/10/1970)	Abasolo municipality	AZ	Silty soil	--	KY034690
22610	E	Sonora	Romo (03/08/1990)	Estación Pesqueira, San Miguel de Horcasitas municipality	--	--	--	KY034688
8423	E	Coahuila	R. Hernández, R López, F. Medrano (09/29/1973)	Hidalgo municipality	AZ	Sandy clay	--	KY034674

Voucher (MEXU)	Clade	Locality	Collector and collection date (mm/dd/yyyy)	Location geography	Type of vegetation	Habitat	Native language	GenBank
8425	E	Coahuila	R. Hernández, R. López, F. Medrano (09/28/1973)	Hidalgo municipality, 150-200 masl	CV	Sandy clay with some grass	--	KY034675
8422	E	Coahuila	R. Hernández, R. López (09/28/1973)	Rancho Palo Blanco, Hidalgo municipality, 147 masl	CV	Sandy clay	--	KY034676
8424	E	Coahuila	R. Hernández, R. López, F. Medrano (09/28/1973)	Rancho Palo Blanco, Hidalgo municipality	CV	Sandy clay	--	KY034677
8426	E	Nuevo León	R. Hernández, R. López, F. Medrano (09/27/1973)	Rancho San José, Anáhuac municipality, 144 masl	CV	Sandy clay	--	KY034679
27843	E	Oaxaca RBTC	A. Medina-Ortiz (09/09/2011)	La Sabana, Santa María Tecomavaca municipality 626 masl, 17°57.501'N, 97°1.266'W	TrDF	Sandy soil, sandy clay, in stony, clayey silt, and cultivation soil	Mazatec and Mixtec	KY034685
21635	N/A	Oaxaca	A. Calderón (07/11/1988)	Zipolite, Puerto Ángel, San Pedro Pochutla municipality	S	Sandy soil	--	N/A
27844	N/A	Oaxaca RBTC	A. Medina-Ortiz, F. Medina-Ruiz (07/13/2013)	Jiotillo redondo, Santa María Tecomavaca municipality 626 masl, 17°57.501'N, 97°1.266'W	TrDF and DS	Sandy clay	Mazatec and Mixtec	N/A
11887	N/A	Oaxaca	O. Téllez (10/24/1977)	San Pedro Totolapam, Oaxaca-Tehuantepec	--	--	--	N/A
1148	N/A	Sonora	E. Matuda (11/22/1962)	Sonoyta, 150 masl	AZ	Sandy clay	--	N/A
22608	N/A	Sonora	M. Esqueda (08/29/ 1988)	Hermosillo	--	Sandy soil	--	N/A

AZ: Arid zone; CV: Chaparral vegetation; DS: Desert scrub; S: Seashore; ThDF: Thorny deciduous forest; TrDF: Tropical deciduous forest. N/A: No sequence data obtained.

(Herrera 1950; Guzmán and Herrera 1969). The collected specimens were deposited in the fungarium of the Herbario Nacional de Mexico (MEXU) of the Instituto de Biología at the Universidad Nacional Autónoma de México (UNAM). In addition, basidiospores we obtained from the center of the dried cap of each of fresh and fungarium fruiting bodies fixed with KOH 5% and photographs were taken (Figure 2 and Suppl. material 1).

DNA extraction, PCR amplification and Sanger sequencing

DNA was extracted from a powder of dried cap (pileus, fresh samples) and the center of the stipe (fungarium samples) from specimens indicated in Table 1. Approximately 5 mg of powdered fungal material was transferred to a bashing bead tube with DNA lysis buffer provided by Zymo research fungal/ bacterial DNA extraction kit. Next, DNA was extracted using the procedures indicated in the Zymo fungal/ bacterial DNA MiniPrep kit.

The entire ITS region was PCR-amplified on an Applied Biosystems Veriti thermal cycler using PuReTaq Ready-To-Go PCR Beads with ITS5 and ITS4 primers (Gardes et al. 1991; White et al. 1990). The PCR reaction was carried out in 25 µL containing 3–5 µL template DNA, 2.5 µL BSA, 2.5 µL 50% DMSO, and 1 µL of each 10 µM forward (ITS5) and reverse (ITS4) primers. Molecular biology grade water from Fisher scientific was added to reach 25 µL. The following thermocycling parameters were used for the amplification: initial denaturation at 95°C for 5 min followed by 39 cycles at 95°C for 30 s, 55°C for 15 s, and 72°C for 1 min, and a final extension step of 10 min at 72°C (Schoch et al. 2012). The PCR products were then examined on an ethidium bromide-stained 1% agarose gel (Fisher Scientific) along with a 1 kb DNA ladder (Promega) to estimate the size of the amplified band. PCR products were purified using a Wizard SV Gel and PCR Clean-up System.

Sanger sequencing of the purified PCR products was performed at Eurofins Genomics (http://www.operon.com/default.aspx) using BigDye Terminator v3.1 cycle sequencing. The sequencing was accomplished bidirectionally using both strands with a combination of ITS5 and ITS4 primers. Sequences were generated on an Applied Biosystems 3730XL high-throughput capillary sequencer. For both sequencing reactions, approximately 15 µL of PCR template were used along with 2 µM sequencing primers.

Phylogenetic analysis

Sequences were assembled with Sequencher 5.3 (Gene Codes), optimized and then manually corrected when necessary; the latter step was to assure that the computer algorithm was properly assigning base calls. Each sequence fragment was subjected to an individual Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) search

in NCBI GenBank to verify its identity. Detailed BLAST search using ITS data were conducted utilizing only published sequences as outlined previously (Raja et al. 2017).

The newly obtained ITS sequences (Table 1) were aligned with ITS sequences of authenticated published sequences or from vouchered fungarium samples (Brock et al. 2009; Osmundson et al. 2013), such as those from a recent phylogenetic study on *Podaxis* spp. (Conlon et al. 2016) using the multiple sequence alignment program MUSCLE (Edgar 2004), with default parameters in operation. *Leucoprinus* was used as an outgroup taxon based on previous studies (Hopple and Vilgalys 1999; Conlon et al. 2016). MUSCLE was implemented using the program Seaview v. 4.3. (Galtier et al. 1996; Gouy et al. 2010). Maximum Likelihood (ML) analysis was perfomed using RAxML v. 7.0.4 (Stamatakis et al. 2008). The analysis was run on the CIPRES Portal v. 3.3 (Miller et al. 2010) with the default rapid hill-climbing algorithm and GTR model employing 1000 fast bootstrap searches. Clades with bootstrap values ≥ 70% were considered significant and strongly supported (Hillis and Bull 1993).

Spore morphology

All spores were measured using a Carl Zeiss Primo Star microscope (Carl Zeiss, Germany) with a Canon PowerShot G6 camera with a Zeiss universal digital camera adapter d30 M37/52′0.75. For each specimen, 25 spores were measured for spore length and width, and presence or absence of a germ pore (Table 2). The Mann-Whitney (U-test) was used to determine whether the mean values of the spore lengths and widths were significantly different between the MEXU specimens assigned to the phylogenetic clades.

Ethnomycological study

This study was conducted in the RBTC, which is located in the states of Puebla and Oaxaca in central Mexico (between 17°32'24" and -18°52'55"N, and 97°48'43" and -97°48'43"W; Figure 1), and its mainly characterized by arid and semiarid vegetation (Valiente-Banuet et al. 2009). This region comprises eight ethnic groups: two in Puebla, the Popolocas and Nahuas; and six in Oaxaca, the Mixtecs, Cuicatecs, Mazatecs, Chinantecs, Chocholtecs, and Ixcatecs (SEMARNAT and CONANP 2013). In the regions where this study was conducted, some people spoke an indigenous language but Spanish was the prevalent mean of communication among them (Table 1). Local people from the RBTC were randomly selected for the ethnomycological interview.

Inhabitants of the region, most 18 years and older, shared their knowledge through the following questionnaire: i) personal information (name, age, sex, ethnicity, place of birth, residence, occupation, and number of family members); ii) knowledge of mushrooms from the locality (traditional name, description of the fruiting body, myths, and uses); iii) how they collect the mushrooms (frequency of collection, if they eat it or buy it); iv) importance of mushrooms in their life; v) how many different mushrooms they

Table 2. Basidiospore measurements.

MEXU	Clade	State	Length ± SD	Ranges	Width ± SD	Ranges	L/W ± SD
1191	D	Oaxaca	11.56 ± 0.71	10 < L < 13	10.96 ± 0.20	10 < W < 11	1.05 ± 0.06
10805	D	Oaxaca	10.92 ± 0.76	10 < L < 13	9.24 ± 0.52	8 < W < 10	1.18 ± 0.06
12808	D	Oaxaca	12.52 ± 1.16	11 < L < 15	9.76 ± 0.93	8 < W < 11	1.29 ± 0.09
7023	D	Oaxaca	10.64 ± 0.70	10 < L < 12	9.68 ± 0.56	9 < W < 11	1.10 ± 0.06
27558	D	Oaxaca	11.28 ± 0.61	10 < L < 12	10.52 ± 0.51	10 < W < 11	1.07 ± 0.05
27845	D	Oaxaca	10.44 ± 0.51	10 < L < 11	9.84 ± 0.47	9 < W < 11	1.06 ± 0.06
27557	D	Oaxaca	10.04 ± 0.68	9 < L < 11	9.20 ± 0.71	8 < W < 10	1.09 ± 0.07
5772	D	Durango	10.80 ± 0.96	9 < L < 13	10.04 ± 0.73	9 < W < 12	1.08 ± 0.06
12338	D	Baja California Sur	11.28 ± 0.89	10 < L < 13	10.52 ± 0.65	10 < W < 12	1.07 ± 0.05
5015	D	Tamaulipas	10.72 ± 0.89	9 < L < 13	9.76 ± 0.60	9 < W < 11	1.10 ± 0.08
7212	D	Tamaulipas	10.32 ± 0.63	9 < L < 12	9.76 ± 0.66	9 < W < 11	1.06 ± 0.06
22610	E	Sonora	15.88 ± 0.97	14 < L < 18	14.00 ± 0.82	13 < W < 16	1.14 ± 0.05
8423	E	Coahuila	14.72 ± 0.79	13 < L < 16	13.88 ± 0.78	13 < W < 16	1.06 ± 0.05
8425	E	Coahuila	14.36 ± 1.22	12 < L < 17	13.28 ± 0.79	12 < W < 15	1.08 ± 0.05
8422	E	Coahuila	14.32 ± 1.03	12 < L < 16	12.76 ± 0.97	11 < W < 15	1.13 ± 0.08
8424	E	Coahuila	14.76 ± 0.60	14 < L < 16	13.68 ± 0.63	13 < W < 15	1.08 ± 0.04
8426	E	Nuevo León	15.36 ± 1.22	13 < L < 18	12.84 ± 0.85	12 < W < 15	1.20 ± 0.08
27843	E	Oaxaca	11.16 ± 0.75	10 < L < 13	10.48 ± 0.59	10 < W < 12	1.07 ± 0.05
21635	N/A	Oaxaca	10.08 ± 0.40	9 < L < 11	9.12 ± 0.73	8 < W < 10	1.11 ± 0.10
27844	N/A	Oaxaca	10.88 ± 0.60	10 < L < 12	10.36 ± 0.49	10 < W < 11	1.05 ± 0.06
10887	N/A	Oaxaca	12.32 ± 1.22	11 < L < 15	10.32 ± 0.63	10 < W < 12	1.19 ± 0.08
1148	N/A	Sonora	16.44 ± 1.04	15 < L < 19	14.20 ± 1.22	12 < W < 17	1.16 ± 0.06
22608	N/A	Sonora	12.28 ± 1.06	11 < L < 15	11.60 ± 1.00	10 < W < 15	1.06 ± 0.06

N/A = Sequence data not available.

see in their locality; vi) if they thought it is important to know the mushrooms; vii) what kind of problems they have when they collect mushrooms in the field; and viii) what information they need to identify the mushrooms.

Results

General morphology of *Podaxis*

All specimens studied (Table 1) share the typical morphological characteristics of the genus *Podaxis* (Figure 2 and Suppl. material 1): white or grayish-white fruit body when young and brown in old or dry specimens, with a long bulbous stem, traversing the gleba as a columella supporting the pileus at the apex. Pileus enveloping a large portion of the stipe, including most of the upper part, with a peridium of two layers and a well-developed capillitium. Exoperidium scaly, most of the scales deciduous at maturity. Endoperidium persistent, membranous, when dehiscing, becoming free

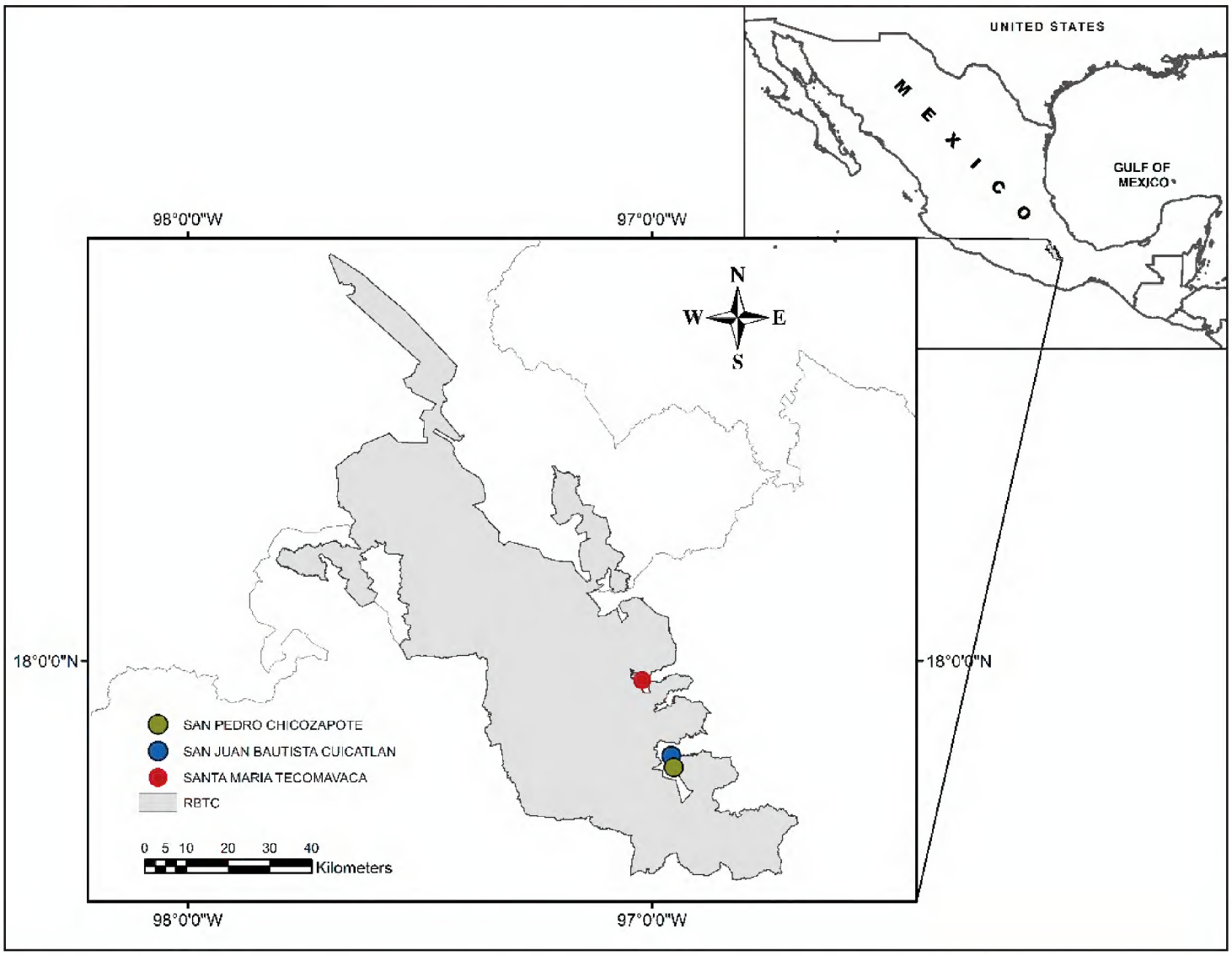

Figure 1. Localities studied in the RBTC. Map was elaborated with ArcGIS.

from the stipe at the base and by longitudinal fissures. Capillitium threads simple, eventually branched and septate, hyaline or pigmented, and flattened. Spores smooth, pigmented, apical pore present, wall of two layers. Basidia fasciculate with 1–4 spores on short sterigmata (Figure 2 and Suppl. material 1).

Variation in basidiospore size and morphology

The length and width, ranges and standard deviation (SD) of basidiospores are outlined in Table 2. MEXU specimens were grouped into two clades (see molecular phylogenetic analysis; Figure 4). Clade D: size of basidiospores in this clade ranged from 9–13 × 8–12 µm (mean = 11–12 × 9–10 µm); and clade E, size of basidiospores in this clade ranged from 9–17 × 9–16 µm (mean = 10–15 × 10–14 µm). Overall the basidiospores in clade D were smaller than basidiospores in clade E (Table 2). Based on the Mann-Whitney (U-test), we found that spore length ($p < 0.001$; Figure 3A) and width ($p < 0.001$; Figure 3B), were significantly different between clades D and E, which supports their molecular phylogenetic placements based on the ITS phylogeny (Figure 4). The color of spores in clade D was generally lighter when compared to those in clade E, which were dark reddish-brown (Figure 2 and Suppl. material 1).

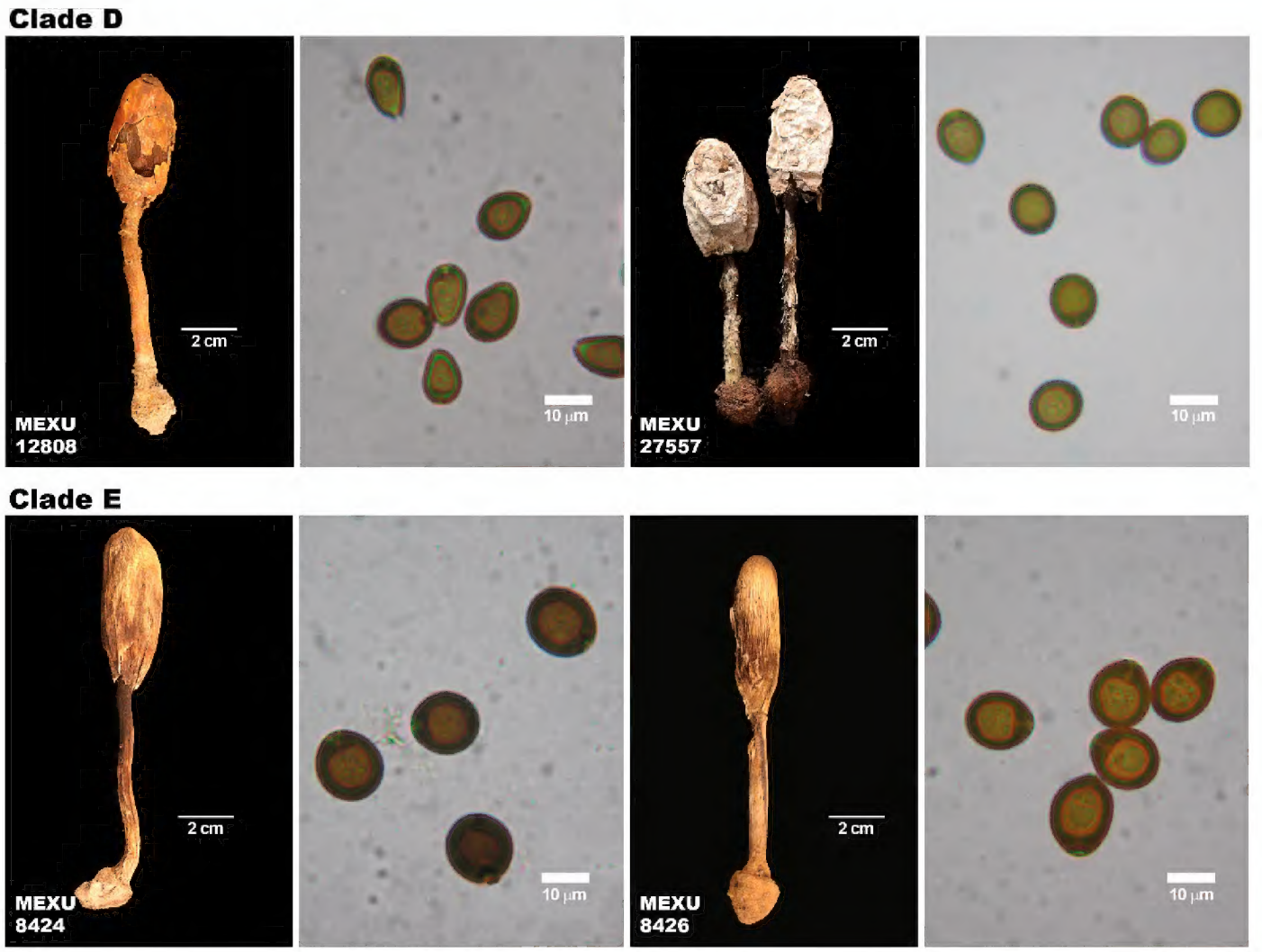

Figure 2. Fruit bodies and basidiospores of selected *Podaxis* specimens from clades D (MEXU 12808 and 27557) and E (MEXU 8424 and 8426).

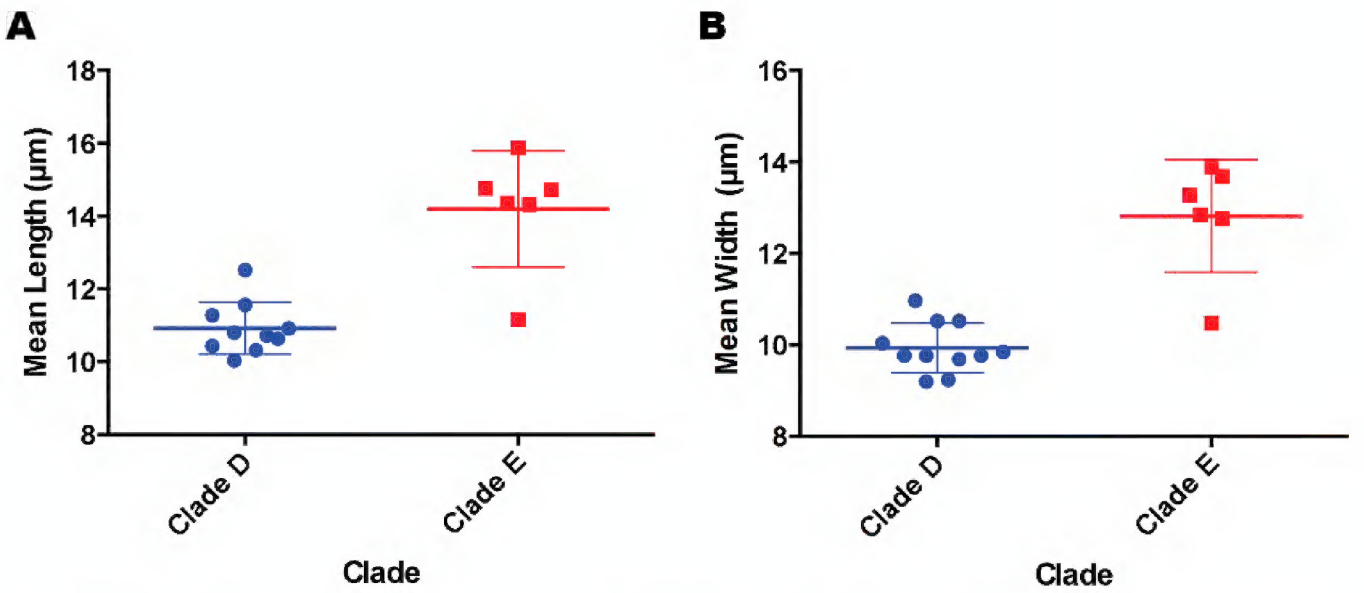

Figure 3. Mean ± SE spore (**A**) length and (**B**) width of MEXU *Podaxis* specimens from clades D and E. Results of Mann-Whitney U test: D–E, $p < 0.001$.

Phylogenetic analysis of molecular data

Eighteen new ITS sequences from different specimens of *Podaxis* from Mexico were obtained; these included four from freshly collected specimens, and 14 from samples in the MEXU fungarium (Table 1). High quality DNA and PCR products were obtained

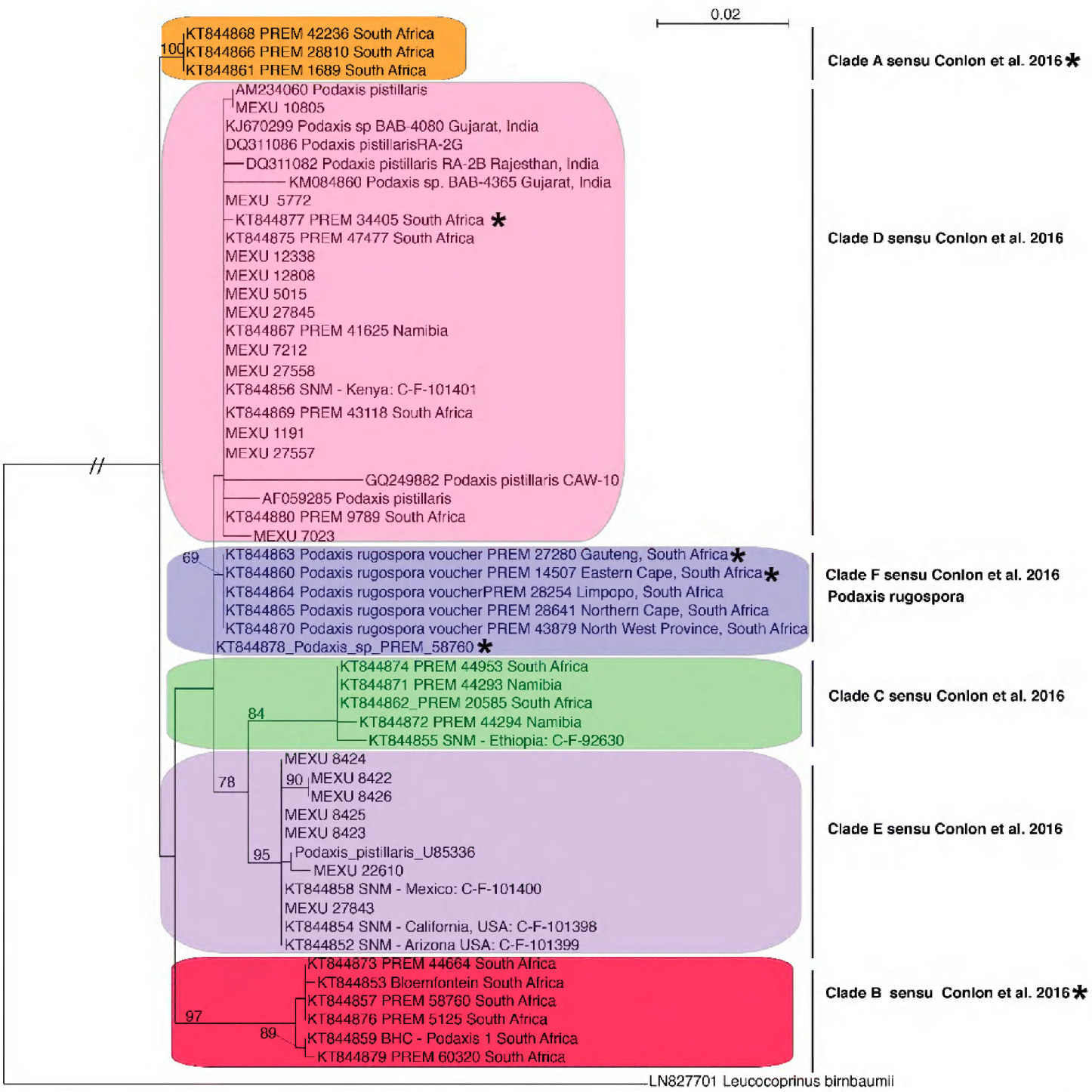

Figure 4. Phylogram of the most likely tree (-lnL = 1860.99) from a RAxML analysis of 56 taxa based on ITS rDNA (681 bp). Numbers above the nodes refer to RAxML bootstrap support values ≥ 70% based on 1000 replicates. Clades to the right (**A–F**) are labeled as per Conlon et al. 2016. The tree is rooted with Leucocoprinus birnbaumii. Symbol (*) next to collections indicates, it was reported from termite mounds. Bar indicates nucleotide substitutions per site.

for all specimens, including MEXU 1191, a collection made in 1948. We were unable to obtain DNA from MEXU 11887, 21635, and 5015 while MEXU 1148 and 27844 produced a PCR band, but resulted in mixed signals perhaps due to low or poor quality of DNA. The ITS alignment consisted of 55 taxa of *Podaxis* and one outgroup taxon (*Leucocoprinus birnbaumii*). The original ITS alignment consisted of 848 nucleotides, after ambiguous regions were limited and removed via GBlocks, the final ITS alignment contained 681 nucleotides.

RAxML analysis of the ITS dataset produced a single most likely tree (Figure 4). We recovered the same six clades (A, B, C, D, E, and F) that were revealed in Conlon

Figure 5. A Desert scrub **B** Immature state of *Podaxis* sp. (MEXU 27843) and **C** at maturity (the gleba changes from white to dark brown (MEXU 27844); Culinary preparation: **D** cleaning process of the fruiting body of *Podaxis* sp. and **E** mixing ingredients for the typical dish [onions, "epazote" (*Dysphania ambrosioides*) and green pepper (chile verde)].

et al. (2016). *Podaxis* spp. from Mexico are phylogenetically placed into two clades (D and E). Seven MEXU specimens (8424, 8422, 8426, 8425, 8423, 22610 and 27843) are placed in (clade E, *sensu* Conlon et al. 2016), with 95% RAxML boostrap support and grouped together with a sequence of *P. pistillaris* (GenBank: U85336), which has been reported in previous molecular phylogenetic studies of Agaricales fungi (Johnson 1999; Vellinga 2004), while 11 other MEXU specimens (10805, 5772, 12338, 12808, 5015, 27845, 7217, 27558, 1191, 27557 and 7023) were nested within clade D (*sensu* Conlon et al. 2016); however this clade did not receive significant RAxML bootstrap support. All ITS sequences generated from this study were deposited in the GenBank and accession numbers are provided in Table 1 (KY034673–KY034690).

Ethnomycology

Ethnomycological importance lies in the fact that people from this region eat the fruiting body of *Podaxis*, commonly known as "hongo" (mushroom), "hongo blanco comestible" (white edible mushroom), or "soldadito" (little soldier), almost daily during rainy season (Figure 5A–C). They cook the mushroom and mix it with green peppers, onions and "epazote" (*Dysphania ambrosioides*), and then make "empanadas" (stuffed

corn tortilla with the mix) (Figure 5D–E). It is considered a tasty mushroom, and according to the habitants of the region, as "one of the tastiest and most nourishing products the land gives us". The local people consider this fungus similar to a "piece of chicken" because of it taste. They also eat it raw, mixed with zucchini, or incorporated in chicken soup and the typical dishes "tesmole", "caldillo" and "mole".

Through the years, the local people have acquired the necessary knowledge to easily locate, harvest and select this mushroom from the land. Although this mushroom is mainly used for personal consumption, some people collect it and sell it in the community. They have also acquired the knowledge about the phenology and ecology of *Podaxis* spp., and they relate the "acidity of rain" with the germination of its spores. In addition, most of the people agree on the following: "when there are constant rains, the fungi starts to grow", "small mushrooms show up after it rains, the sun comes out and the sky is clear", "in order for it to grow, the mushroom needs sunlight for one or two days". Concerning the habitat and soil, they indicate that: "mushrooms grow mainly on the river bank or on sandy soil" but also "mushrooms are produced throughout the mountain slopes, even on agricultural production areas". They also say: "if you find one, you will find two" or "they are born in pairs". Finally, when a mushroom fruiting body has "aged", the local people spread the spores in places where they want the fungi to grow next rain season, and they say: "if they don't grow this season, they'll grow during the next one".

Discussion

Phylogenetic affiliations of MEXU specimens based on molecular and morphological comparison

Podaxis pistillaris sensu lato has been collected and reported from numerous semi-arid regions around the world, fruiting mainly in the rainy seasons. In Mexico, it has widely been collected from north to south (Herrera 1950; Dennis 1960; Guzmán and Herrera 1973; Urista et al. 1985; Aparicio-Navarro et al. 1991). Despite its wide geographical distribution, the identification of *P. pistillaris* remains confusing mainly because the type specimen of *P. pistillaris* collected and described from India has not been sequenced (Linnaeus 1771), making a true molecular phylogenetic assessment difficult. It is likely that cryptic speciation is rampant in this widely distributed species.

All the studied specimens from the MEXU fungarium were named as *P. pistillaris* based on its morphological characteristics (Figure 2 and Suppl. material 1); however, molecular phylogenetic analysis of the ITS region of these specimens, along with ITS sequences from a recent study of *Podaxis* spp. from South Africa (Conlon et al. 2016), placed the MEXU specimens into two clades: D and E (Figure 4). Therefore, our analysis indicate there are at least two phylogenetic species of *Podaxis* in Mexico, and not all species of *Podaxis* collected from Mexico should be identified as *P. pistillaris.*

Interestingly, all specimens in clade E (Figure 4) have been reported from North America, including Mexico. In our ITS phylogeny seven MEXU specimens (8424, 8422, 8426, 8425, 8423, 22610 and 27843) were grouped with an ITS sequence of *P. pistillaris* (GenBank: U85336; Johnson 1999; Vellinga 2004) with significant bootstrap support (95%). However, at this time it is not possible to name this clade. This is because there are other species from the new world, including southwestern US, Mexico and Argentina such as *P. argentinus* Speg., *P. longii* McKnight, *P. microporus* McKnight (McKnight, 1985), *P. farlowii* Massee (Morse 1933), and *P. mexicanus* (Ellis 1893), which need to be examined in light of molecular phylogenetic analysis.

Clade E (Figure 4) is entirely comprised of specimens from the new world and all of these occur as free-living in desert-like semi-arid regions (Table 1). There have been reports of symbiotic association of *Podaxis* spp. with termites in Australia (Priest and Lenz 1999; Young et al. 2002), Nigeria (Alasoadura 1966), South Africa (Bottomley 1948; Conlon et al. 2016), and Bolivia (Rocabado et al. 2007). In this context, it is worth to mentioning that in the RBTC (Oaxaca, Mexico) such a symbiotic association with termites has not yet been reported. Further molecular studies of *Podaxis* specimens collected from the new world are required to test the hypothesis of loss or gain of termite symbiosis in this clade.

We examined the spore sizes and morphology of MEXU specimens from clade E and compared them to the measurements obtained from the type material of *P. pistillaris* in the LINN fungarium (Priest and Lenz 1999). The spore size of 10–14 × 9–12 µm from the type material fits the average measurements (10–15 × 10–14 µm) obtained from the MEXU specimens in clade E (Table 2 and Figure 3). The spore color of most specimens in clade E is also reddish-brown with thick-walls (Figure 2 and Suppl. material 1). These attributes are in agreement with the type specimen examined by Priest and Lenz (1999). However, the type specimen from the LINN herbarium needs to be sequenced to corroborate the morphological data.

Eleven of the eighteen specimens (10805, 5772, 12338, 12808, 5015, 27845, 7217, 27558, 1191, 27557, and 7023) were nested within clade D (*sensu* Conlon et al. 2016), but without significant RAxML bootstrap support (Figure 4). Other members in clade D include seven sequenced specimens from GenBank both labeled as *Podaxis* sp. and/or *Podaxis pistillaris* and mostly included specimens collected from desert-like arid regions in western India (Singh et al. 2006). When we removed all other GenBank data from our analysis and only included sequences from our study and those of Conlon et al. (2016), we found that clade D had significantly high bootstrap support (82%; data not shown). All specimens from clade D were reported to be free-living with the exception of PREM 34405 from South Africa (Conlon et al. 2016). The average spore measurements of MEXU specimens in clade D were 11–12 × 9–10 µm (Table 2 and Figure 3), which was well within the range of those reported in clade D by Conlon et al. (2016). Specimens in clade D were reported from both the New World (MEXU) and the Old World (South Africa and India), suggesting that species in this clade are widely distributed geographically.

Based on the fruiting body morphology, it was difficult to separate MEXU specimens in clade D and E (Figure 2 and Suppl. material 1). This observation agrees with the results from Conlon et al. (2016) as they reported that fruiting body morphology of *Podaxis* spp. does not significantly differ between the termite associated and free-living clades. The spores in clade D (free-living and termite associated) and E (free-living only) were both thick-walled (Figure 2 and Suppl. material 1); this result agrees with the observations made by Conlon et al. (2016), who reason that free-living, desert dwelling species have thick-walled spores as it may help prevent desiccation in desert-like dry environments. Due to the lack of molecular data from type specimens except for *P. rugospora* (Conlon et al. 2016), currently it is not possible to name specimens in either clade D or E. Based on our preliminary molecular phylogenetic analysis of MEXU specimens, it seems highly unlikely that all MEXU specimens represent *P. pistillaris.*

Habitat and geographical distribution

Podaxis species in Mexico are found predominately in open areas, growing solitary in sandy or clay soils of arid and tropical zones (Table 1). They have been found in the states of Baja California, Durango, Nuevo León, Tamaulipas, Oaxaca (Ruiz-Oronoz and Herrera 1948; Herrera 1950; Guzmán and Herrera 1973), Mexico City (Dennis 1960), Coahuila (Urista et al. 1985), Chihuahua (Moreno et al. 2010) and Sonora (Ellis 1893; Aparicio-Navarro et al. 1991). They have also been reported from the USA (Oregon, California, Arizona, Nevada, New Mexico, Texas, and Hawaiian) (Brasfield 1937; Keirle et al. 2004), Jamaica (Dennis 1953), Galapagos islands (Reid et al. 1980), Argentina (Martínez 1971), Brazil (Baseia and Galvão 2002), Bolivia (Rocabado et al. 2007), Asia (Sinai Peninsula, Israel, Saudi Arabia, Afghanistan, Iran, Pakistan, Kuwait, Qatar, India, China) (Morse 1941; Dring and Rayss 1963; Binyamini 1973; Watling and Gregory 1977; Patel and Tiwari 2012; Muhsin et al. 2012; Mahmoud and Al-Ghamdi 2014), Africa (Madagascar South, Congo, Nigeria, South Africa) (Dissing and Lange 1962; Bottomley 1948; Dring 1964; Alasoadura 1966; Conlon et al. 2016), and Australia (Hilton and Kenneally 1981; Priest and Lenz 1999; Young et al. 2002).

Ethnomycology

In Mexico, the use of *Podaxis* species for food consumption has not been reported. Our study includes data from interviews that state the consumption and farming of this mushroom within the RBTC. In this area, the species is greatly valued by the local people, who sell the fungus for 1–1.5 USD per kilogram or consume young fruiting bodies of *Podaxis* in typical dishes from the region, particularly as "empanadas" (Figure 5), a favorite among the people of the region. They also have developed the ability to locate and harvest the mushrooms, as well as farming (proto-cultivation) is considered very important during rainy/wet season. To consistently obtain more fruiting bodies,

the locals scatter the spores in the soil where they want the fungus to grow and emerge in the following wet season. This method of spore spreading helps them to locate and collect the mature fungus more quickly.

On the other hand, *Podaxis* has also been catalogued as a non-edible mushroom (Guzmán 1977) and has been referred as being toxic in Nigeria and South Africa (Alasoadura 1966); contrastingly, it has been reported as edible in Afghanistan, Pakistan, India, and Australia (Batra 1983). People from the Sind Province of Pakistan are familiar with the commerce of "Khumb" or "Khumbi" (fungus *P. pistillaris*). Khumbi is also a term used by rural communities in Haryana, India, who also refer to this fungus as "Saanp ki chhatri" (umbrella of a snake or snake's cap) (Mridu and Atri 2015). In this region, the mushroom is much appreciated as it is considered a delicacy with medicinal properties for the "Hakims", the dispensers of folk medicine (Khan et al. 1979).

The names attributed to this species in the three communities of the RBTC are "hongo" (mushroom), "hongo blanco comestible" (white edible mushroom) and "soldadito" (little soldier) (Table 1). This fungus is known as "black powderpuff" in Australia (Grey and Grey 2005), "desert shaggy mane" in Pakistan and the USA (Yousaf et al. 2013; Hopple and Vilgalys 1999), "Khumbi" in the India, "Al-Arjoon" in Saudi Arabia, Kuwait and Qatar; "Kama" in Iraq (Muhsin et al. 2012; Mahmound and Al-Ghamdi 2014), and as "Faswat imgaar", "Faswat al-awzaiq", and "Faswat al-dheib" in Yemen (Kreisel and Al-Fatimi 2004).

In Yemen and South Africa, *Podaxis* is used for its medicinal properties and antibacterial activity against *Staphylococcus aureus*, *Micrococcus flavus*, *Bacillus subtilis*, *Serratia marcescens*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* (Al-Fatimi et al. 2006; Panwar and Purohit 2002). In Australia, it has been used as hair dye (Batra 1983), while in West Africa, *P. pistillaris* is used to produce baby-powder (desiccative) as an anti-abortive (Gérault and Thoen 1992). Such medicinal properties arise from the chemical constituents of the fruiting body, which include nitrogen, crude protein, true protein, carbohydrates, lipids, and ash content (Gupta and Kapoor 1990; Gupta and Singh 1991; Khaliel et al. 1989 and 1991).

Conclusions

Podaxis is considered a very important mushroom in arid regions of the world due to its culinary and medicinal values. Further taxonomic and molecular phylogenetic studies of this genus are urgently required to better understand species boundaries and provide accurate names on specimens of *Podaxis,* particularly the ones used as food in Mexico and worldwide. Better understanding of *Podaxis* spp. might be possible when mycologists work closely with local communities in different parts of both the Old and New World. Our study provides preliminary morphological and molecular data from *Podaxis* specimens collected in Mexico along with its ethnomycolgy use. We anticipate our study will encourage future phylogenetic diversity analyses on this widely distributed yet taxonomically poorly studied genus of Agaricomycetes.

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Supplementary material 1

Fruit bodies and basidiospores of *Podaxis* specimens

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